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SUSCEPTIBILITY

In Vitro Activities of Eravacycline and Other Antimicrobial Agents against Human Mycoplasmas and Ureaplasmas

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ABSTRACT We performed *in vitro* susceptibility testing for eravacycline in comparison to 4 other antimicrobials against 10 *Mycoplasma genitalium*, 40 *Mycoplasma hominis*, 44 *Mycoplasma pneumoniae*, 20 *Ureaplasma parvum*, and 20 *Ureaplasma urealyticum* isolates. All eravacycline MICs were ≤0.25 μg/ml, except that for one isolate of *M. genitalium*, for which the MIC was 2 μg/ml. Eravacycline was markedly more potent than tetracycline, azithromycin, moxifloxacin, and clindamycin against all isolates tested, which included 37 macrolide, tetracycline, and/or fluoroquinolone-resistant organisms.

KEYWORDS eravacycline, mycoplasma, pneumonia, ureaplasma, urogenital infection

Eravacycline (TP-434) is a synthetic halogenated tetracycline derivative that is undergoing clinical development by Everest Medicines and has activity against a broad spectrum of microorganisms, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* species, and carbapenem-resistant Gram-negative bacilli (1). Although the eravacycline binding site is similar to that of tetracycline, a significant advantage is that it retains activity against organisms with the two main tetracycline resistance mechanisms, efflux and ribosomal protection (1).

Mycoplasma pneumoniae is a cause of tracheobronchitis and community-acquired bacterial pneumonia (CABP) in children and adults (2). *Mycoplasma hominis* and *Ureaplasma* spp. cause a variety of urogenital conditions in adults and are also systemic pathogens in infants (3). *Mycoplasma genitalium* is an important cause of male urethritis, female cervicitis, and pelvic inflammatory disease, and it may also be associated with adverse pregnancy outcomes (4, 5). Invasive disease may occur in persons who are immunosuppressed, particularly in the setting of defects in humoral immunity or organ transplantation (2, 3). Treatment of *M. pneumoniae* infections has been complicated by macrolide resistance mediated by mutations in 23S rRNA that has spread worldwide over the past 2 decades (2, 6). Tetracycline resistance often occurs in *M. hominis* and *Ureaplasma* species due to ribosomal protection mediated by the *tetM* transposon (7). Resistance to macrolides due to mutations in 23S rRNA and resistance to to fluoroquinolones due to mutations in *parC* and/or *parE* have been documented in these organisms, as well as in *M. genitalium* (7, 8). Tetracyclines have historically not worked well for treatment of *M. genitalium* infections, but high-level resistance has not previously been documented in this species, and no isolates described thus far have been shown to contain *tetM* (9). Persons who are immunosuppressed and those who have received numerous courses of antimicrobials are at greater risk for having systemic infections with drug-resistant organisms (7). For these reasons, new agents that are not affected by cross-resistance to other drug classes are needed for treatment of *Mycoplasma* and *Ureaplasma* infections. There are no published data concerning the *in vitro* activity of eravacycline against *Mycoplasma* or *Ureaplasma* species.

We performed an *in vitro* study testing eravacycline against a collection of *Myco-*

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TABLE 1 MIC summary

Organism or drug	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Mycoplasma genitalium ($n = 10$)			
Eravacycline	0.002 to 2	0.008	0.125
Tetracycline	0.125 to 32	0.25	4
Azithromycin	$\leq$0.001 to 32	$\leq$0.001	4
Moxifloxacin	0.032 to 8	0.063	2
Mycoplasma hominis ($n = 40$)			
Eravacycline	0.002 to 0.008	0.004	0.008
Tetracycline	0.016 to 32	0.25	16
Clindamycin	0.008 to >32	0.063	0.063
Moxifloxacin	0.032 to 8	0.063	0.125
Mycoplasma pneumoniae ($n = 44$)			
Eravacycline	0.004 to 0.008	0.008	0.008
Tetracycline	0.25 to 0.5	0.5	0.5
Azithromycin	$\leq$0.0005 to >32	$\leq$ 0.0005	32
Moxifloxacin	0.125	0.125	0.125
Ureaplasma species ($n = 40$)			
Eravacycline	0.032 to 0.25	0.125	0.25
Tetracycline	0.125 to >32	0.5	8
Azithromycin	0.5 to >32	2	8
Moxifloxacin	0.125 to 8	0.5	2

plasma and *Ureaplasma* spp. obtained from across the United States, Europe, and China. Organisms included 44 clinical isolates of *M. pneumoniae* (obtained since 2008), 40 isolates of *M. hominis* (30 obtained since 2015), 20 isolates of *Ureaplasma parvum*, 20 isolates of *Ureaplasma urealyticum* (37 obtained since 2013), 10 *M. genitalium* isolates with 2 of them obtained since 2018, and 8 older clinical isolates and reference strains. Organisms included 37 isolates resistant to tetracyclines, macrolides, or fluoroquinolones, alone or in combination. With the exception of 2 recent *M. genitalium* isolates from the same patient, all other isolates were from nonduplicate patients. A complete listing and description of all bacterial isolates tested along with the individual MICs for each of them is provided as Table S1 in the supplemental material. Comparator agents were tetracycline, azithromycin, and moxifloxacin. Clindamycin was substituted for azithromycin for *M. hominis* due to intrinsic resistance to 14- and 15-membered macrolides (10). Antimicrobial agents were obtained in powdered forms of known purity and dissolved in accordance with their manufacturer's instructions. Inoculum was prepared, then MICs were determined and interpreted using the broth microdilution technique in accordance with Clinical and Laboratory Standards Institute (CLSI) guideline M43-A (11). Organisms were stored frozen at −80°C until thawing for testing. Table 1 summarizes MIC data.

Eravacycline was the most active drug overall against each species. All eravacycline MICs were $\leq$0.25 μg/ml, with the exception of that of one *M. genitalium* isolate, which was 2 μg/ml. The eravacycline MIC_{90} (0.125 μg/ml) for *M. genitalium* was 16-fold lower than that for moxifloxacin (2 μg/ml) and 32-fold lower than those of tetracycline and azithromycin (4 μg/ml). Older strains of *M. genitalium* had much lower MICs for eravacycline (MIC_{50} = 0.008 μg/ml) and tetracycline (MIC_{50} = 0.25 μg/ml).

All eravacycline MICs tested against *M. hominis* were $\leq$0.008 μg/ml. The MIC_{90} (0.008 μg/ml) was 8-fold lower than that for clindamycin (0.063 μg/ml) and 16-lower than that for moxifloxacin (0.125 μg/ml). This collection included one *M. hominis* isolate resistant to clindamycin (MIC, >32 μg/ml), as well as 8 isolates resistant to tetracycline (MICs, 8 to 32 μg/ml), and 3 isolates resistant to moxifloxacin (MICs, 1 to 8 μg/ml). Two isolates were resistant to both of these drugs. Eravacycline MICs were unaffected by resistance to clindamycin, tetracycline, and moxifloxacin.

Eravacycline was active against *M. pneumoniae* with all MICs being $\leq$0.008 μg/ml. The MIC_{90} (0.008 μg/ml) was 16-fold lower than that for moxifloxacin (0.125 μg/ml) and

64-fold lower than that for tetracycline (0.5 μg/ml). Azithromycin was the most potent agent against 30 macrolide-susceptible isolates (MICs, $\leq$0.0005 μg/ml), whereas the MICs for 14 macrolide-resistant isolates were 8 to 32 μg/ml.

Eravacycline MICs against *Ureaplasma* species were all $\leq$0.25 μg/ml. The MIC_{90} (0.25 μg/ml) was 8-fold lower than that for moxifloxacin (2 μg/ml) and 32-fold lower than those for azithromycin and tetracycline (8 μg/ml). There was no difference in activity of any antimicrobials against *U. parvum* versus *U. urealyticum*. This collection included 4 isolates of *U. parvum* and 7 isolates of *U. urealyticum* with macrolide resistance (azithromycin MICs of $\geq$32 μg/ml), tetracycline resistance (MICs, 8 to $>$32 μg/ml), and/or fluoroquinolone resistance (moxifloxacin MICs, 4 to 8 μg/ml). One isolate of *U. urealyticum* was resistant to both tetracycline and moxifloxacin, and another was resistant to tetracycline and azithromycin. Eravacycline MICs were unaffected by macrolide, tetracycline, or fluoroquinolone resistance.

Eravacycline is a fluorocycline that is structurally similar to tigecycline with modifications to the D ring of its tetracycline core, in which a fluorine atom replaces the dimethylamine moiety and a pyrrolidinoacetamido group replaces the 2-tertiary-butyl glycylamido (12). In 2018, the FDA granted approval for an intravenous formulation of eravacycline for treatment of complicated intra-abdominal infections (CIAI) in adults. Eravacycline and omadacycline, another synthetic tetracycline derivative, both have a broad spectrum of activity according to *in vitro* and clinical studies, encompassing many types of Gram-positive and Gram-negative pathogens, including those with multidrug resistance mechanisms (1, 12, 13).

Our previous studies have demonstrated good activity *in vitro* for omadacycline (8) as well for as another fluorocycline, TP-271 (14) against *M. pneumoniae*. The TP-271 MIC_{90} was 0.004 μg/ml (range, 0.0005 to 0.008 μg/ml) (14), similar to what we observed for eravacycline, for which MICs ranged from 0.004 to 0.008 μg/ml with an MIC_{90} of 0.008 μg/ml. Our prior experience with omadacycline (8) showed that MICs for *M. pneumoniae* ranged from 0.125 to 0.25 μg/ml with an MIC_{90} of 0.25 μg/ml, signifying that its activity was similar to those of tetracycline and doxycycline, for which MIC_{90} values were 0.5 μg/ml. A previous report in which *M. pneumoniae* was tested against tigecycline indicated that its activity was similar to that of tetracycline, with MICs ranging from 0.06 to 0.25 μg/ml and an MIC_{90} of 0.25 μg/ml (15). Although there have been few direct comparisons of omadacycline versus eravacycline, one study reported lower MICs for eravacycline against *Mycobacterium abscessus* (16), consistent with our observation for *M. pneumoniae*. There are no FDA or CLSI breakpoints for mycoplasmas or ureaplasmas for eravacycline. However, FDA breakpoints are $\leq$0.06 μg/ml for *Staphylococcus aureus*, *Enterococcus* spp., and *Streptococcus anginosus*. A breakpoint of $\leq$0.5 μg/ml has been designated for *Enterobacteriaceae* species and certain anaerobes. Using these breakpoints as a general guide, eravacycline MICs for *M. pneumoniae* were below these values for designating susceptibility. Previous studies have also shown eravacycline to be highly active against other respiratory pathogens, including *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Legionella pneumophila* (1), suggesting a potential role in treatment of CABP.

Urogenital *Mycoplasma* and *Ureaplasma* species have more diverse antimicrobial resistance patterns than *M. pneumoniae*, for which naturally occurring resistance is limited to macrolides (7). The present study confirmed that all *M. hominis* isolates, including those with resistance to clindamycin, tetracycline, and moxifloxacin, were inhibited by eravacycline at concentrations of $\leq$0.008 μg/ml, well below the FDA MIC breakpoint for susceptibility for Gram-positive bacteria. Our previous study (8) found that omadacycline MICs for *M. hominis* ranged from 0.016 to 0.125 μg/ml. The MIC_{90} was 0.063 μg/ml, 8-fold higher than that for eravacycline found in the present study (0.008 μg/ml).

Ureaplasma spp. often have higher MICs than *M. hominis* for tetracyclines and fluoroquinolones, even in the absence of acquired resistance genes. This was evident in the present study, as indicated by MIC_{50} values, which were 2-fold higher for tetracycline (0.5 versus 0.25 μg/ml) and 8-fold higher for moxifloxacin (0.5 versus

0.063 μg/ml). Our earlier data for omadacycline (8) indicated that it was less potent against *Ureaplasma* spp. than eravacycline, with MICs ranging from 0.25 to 2 μg/ml and an MIC_{90} of 2 μg/ml. Most eravacycline MICs for *Ureaplasma* spp. were higher than the FDA breakpoint for susceptibility for Gram-positive bacteria but were lower than the 0.5 μg/ml cutoff designated for *Enterobacteriaceae* and anaerobes.

M. genitalium urogenital infections can be difficult to treat, and the organisms may rapidly develop resistance to macrolides and fluoroquinolones (9). Tetracycline MICs are generally less than 0.5 μg/ml (7), but we encountered an isolate from the urine of a man with congenital hypogammaglobulinemia who had failed multiple antimicrobial treatment courses, including one of doxycycline, for which the tetracycline and azithromycin MICs were 4 μg/ml, moxifloxacin MIC was 2 μg/ml, and eravacycline MIC was 2 μg/ml. Another isolate from this same man had an eravacycline MIC of 0.125 μg/ml with elevated MICs for azithromycin and tetracycline (32 μg/ml) and moxifloxacin (8 μg/ml). Genome sequencing revealed multiple point mutations in an efflux ABC transporter, suggesting that the function of this efflux transporter may be affected and contribute to the resistance. There was also a single-nucleotide polymorphism in the 16S rRNA gene with unknown impact. There was no evidence of *tetM* in this strain. This potential new mechanism of tetracycline resistance in *M. genitalium* that also affected eravacycline to some extent will be fully described in a separate publication. Eravacycline resistance in some other bacteria is associated with upregulated, nonspecific intrinsic efflux and with target site modifications such as to the 16S rRNA or to certain 30S ribosomal proteins (17, 18), so our findings in *M. genitalium* are new, but not completely unexpected.

Overall, these data suggest eravacycline, in addition to its already approved CIAI indication, may be a useful alternative for treating some respiratory and/or urogenital infections caused by human mycoplasmas and ureaplasmas, including infections caused by macrolide, tetracycline, and fluoroquinolone-resistant organisms, thereby justifying further clinical studies. Availability of an oral formulation will greatly enhance such a possibility. We have also demonstrated that overt tetracycline resistance can occur in *M. genitalium*, presumably as a result of antimicrobial selective pressure, and is perhaps mediated by an efflux mechanism that can also affect eravacycline activity to some extent.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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